Molecular Imaging and Atherosclerosis

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Introduction

One emerging technology in MR molecular imaging is its ability to detect unique biochemical activities for the in-vivo diagnosis of pathological processes. The ability to target specific molecules of plaques may greatly enhance detection and characterization of atherosclerotic and atherothrombotic lesions (**Figure 1**) using MRI[1]. Atherosclerotic plaque components may be differentiated by introduction of plaque-specific contrast agents related to molecular signatures involved in the disease process. Another strategy is to link the contrast agents to antibodies[2-5] or peptides[6, 7] that target specific plaque components or molecules that localize to specific regions of atherosclerotic plaques.[8-11] Cellular targeting of mononuclear cells such as monocytes, macrophages, and foam cells is an attractive means of identifying atherosclerosis since these cells have been shown to play a pivotal role in the progression of atherosclerosis [12, 13]. At present, macrophages have only been imaged with iron oxide compounds (USPIOs, SPIOs) that are removed from the circulation by macrophages and other cells of the reticuloendothelial system[9-11]. In a study by Kooi et al. on 11 symptomatic patients scheduled for carotid endarterectomy, 75% of ruptured or rupture-prone lesions demonstrated uptake of USPIOs compared with only 7% of stable lesions and a decrease in signal intensity of 24% on T2*[10].

Current research is ongoing to determine the uptake of target macrophages with gadolinium-based contrast agents linked in the form of immunomicelles (micelles containing an antibody) that target the macrophage scavenger receptor in vitro. Results showed a 59% increase in signal intensity in the aortas incubated with immunomicelles compared to a 19% increase in the control. Preliminary in-vivo studies in an ApoE knockout mouse showed uptake of a fluorescently labeled immunomicelles by resident macrophages in the plaque. This is the first study to target macrophages with paramagnetic immunomicelles. This biological activity imaging

approach may ultimately become useful *in vivo* to detect the high macrophage density characteristic of the high-risk plaques.

Neovascularization has been shown to play an important role in atherosclerosis and the integrin $\alpha_v\beta_3$ has been targeted to identify regions in the vessel wall undergoing neovascularization [3-5]. Winter et al. recently demonstrated in a rabbit model of atherosclerosis that regions of neovascularization in plaque had a 47% increase in signal intensity following treatment with $\alpha_v\beta_3$-targeted nanoparticles[5].

Potential targets of interest for imaging atherosclerotic plaque with molecular specific MR contrast agents includes oxidized low-density lipoprotein (oxLDL), tissue factor, endothelial integrins, matrix metalloproteinases, and extracellular matrix proteins discussed extensively in a recent paper by Choudhury et al.[14] and are listed in **Table 3**. While targeted nuclear imaging through the use of antibodies specific to oxLDL has been demonstrated promises at detecting atherosclerosis[15], estimating plaque volume[16], and following progression/regression of atherosclerosis[17], we are unaware of any studies evaluating oxLDL as a target for molecular MRI of atherosclerosis.

Additionally, molecules such as tissue factor[18] and endothelial integrins[18-20] such as E-selectin, P-selectin, ICAM-1, or VCAM-1 have been targeted with antibodies linked to echogenic contrast agents. While these agents utilize echocardiography or nuclear imaging, the echogenic or nuclear contrast agents could easily be replaced by linking an MR contrast agent to the monoclonal antibody to target the molecule of interest. Ultimately, the identification of molecules found only in atherosclerotic plaque will enable improved detection of atherosclerotic plaque assuming that the target is expressed in adequate quantity for detection by molecular MRI.

The ability to identify components of thrombus with molecular MRI may enable enhanced detection and characterization of both luminal thrombus and components of an organized

thrombus in an old atherothrombotic lesion. Therefore, selection of targets in the coagulation cascade, such as fibrin, factor XIII, integrins on the surface of platelets, and tissue factor, is vital in identifying the areas of active or old thrombus formation. Monoclonal antibodies or peptide ligands with an attached MR contrast agents that specifically bind to components of thrombus have been performed in animal models[7, 21-23] and humans[24, 25]. Additionally, thrombus resulting from plaque rupture has been identified using fibrin-specific MR contrast agents in a rabbit carotid crush injury model[6, 26]. In the 25 arterial thrombi induced by carotid crush injury, Botnar et al. demonstrated a sensitivity and specificity of 100% for in vivo thrombus detection using MRI[6]. Sirol et al.[26] recently investigated a similar fibrin-specific MR contrast agent in 12 guinea pigs demonstrating that thrombus signal intensity was increased by over 4-fold after intravascular delivery of contrast agent and thrombus was detected in 100% of animals post-contrast compared with 42% identification of thrombus pre-contrast[26].

A new class of contrast agents, called Gadofluorines is based on a macrocyclic, lipophilic gadolinium chelate complex (1,528Da) with a perfluorinated side chains forms micelles in aqueous solution with a potential for plaque enhancement. Initial studies have demonstrated the application of Gadofluorines as a lymphographic contrast media Studies done on rabbits showed enhancement of the agent on atherosclerotic plaque although has been limited in determining the uptake of the agent on a particular plaque component Sirol et al.[110] and others[27] demonstrated that Gadofluorine M enhanced aortic wall imaging in New Zealand White (NZW) rabbits but did not enhance the aorta of control rabbits. Gadofluorine M increased signal intensity by 164% at 1-hour post-contrast and increased signal intensity 207% at 24 hours post-contrast[8]. A strong correlation was found between the lipid-rich areas in histological sections and signal intensity in corresponding MR images[8] and suggests a high affinity of Gadofluorine M for lipid-rich plaques

(**see Figure 2**). Further studies done on the ApoE knockout mice showed it to be co-localized in the extracellular matrix in the atherosclerotic plaque. **(Figure 3).**

Another novel imaging agent that is being developed is using a recombinant high-density lipoprotein (rHDL) molecule that incorporates gadolinium-DTPA phosholipids[28]. High-density lipoproteins present in the plasma play a key role in reverse cholesterol transport by removing excess cellular cholesterol from the peripheral tissues. This imaging agent several advantages; it has a diameter of 7-12 nm, is endogenous, does not trigger an immune reaction, and is easy to reconstitute[28]. This agent was tested in-vivo in Apolipoprotein E (ApoE) knockout mice and demonstrated a 35% mean normalized enhancement ratio in the atherosclerotic plaque 24 hours following intravascular injection and a significant uptake of fluorescently labeled rHDL and imaged by confocal microscopy[28]. **Figure 4** demonstrates the enhancement of the plaque with rHDL and provides an illustration of the contrast agent.

Future and Conclusions:

Further improvements in MR coronary plaque imaging [29] is on the horizon and would solidify the evaluation of simultaneous multi-vessel (aorta, coronary arteries, carotid arteries, and other peripheral arteries) assessment of atherosclerosis. Future work will be geared towards a 3.0T or higher whole-body MR systems. New black blood techniques have recently been introduced for the simultaneous acquisition of multiple slices and shown to greatly reduce total examination time [30, 31]. Prospective studies are needed to determine the predictive value of fibrous cap characteristics of atherosclerosis, as visualized by MR, for risk of subsequent ischemic events.[32]

MR use for serial monitoring of atherosclerotic plaque progression and regression in the carotid arteries and aorta is clearly progressing rapidly, and may be the noninvasive imaging technology of choice for this purpose in the future, given the high image quality and the

sensitivity to small changes in plaque size and possible characterization.

Combining different imaging modalities and adding new biomarkers of disease such as MR molecular targeting that can be detected non-invasively may be necessary to grasp the full picture of the disease, aid in the diagnosis, risk stratification and management and may help predict future cardiovascular events.

Table 1 | Molecular magnetic resonance imaging

Target	Ligand	Agent	Disease/ process	Model	Field strength*	Maximum spatial resolution‡
ICAM1	Anti-ICAM1 antibody	Antibody-conjugated paramagnetic liposome (ACPL)	Encephalitis	Mouse brain *ex vivo*	9.4	0.04 × 0.04 × 0.04
E-selectin	Sialyl Lewis mimetic	Gd-DTPA-B(sLEx)	Multiple sclerosis	Rat brain *in vivo*	7	0.23 × 0.23 × 2
α v β3 integrin	DM 101 antibody	Gd-perfluorocarbon nanoparticles	Corneal angiogenesis	Rabbit *in vivo*	4.7	0.2 × 0.1 × 2
α v β3 integrin	RGD peptide vitronectin antagonist	Peptide-conjugated LEFPC	Atherosclerosis	Rabbit aorta *in vivo*	1.5	0.25 × 0.23 × 5
αIIbβ3	RGD cyclic peptide	RGD-USPIO	Platelet thrombus	Pig vein *in vivo*	1.5	0.3 × 0.3 × 1
Fibrin	Antifibrin monoclonal antibody	Paramagnetic nanoparticles	Thrombus	Human thrombus *ex vivo*	4.7	0.05 × 0.05 × 0.5
Fibrin	Antifibrin F(ab)	F(ab) monoclonal LEPFC	Venous thrombus	Dog *in vivo*	1.5	0.9 × 0.7 (in plane)

*Measured in Tesla. ‡Resolution given in mm3. DTPA, diethylene triamine pentaacetic acid; F(ab), antigen-binding fragment; Gd gadolinium; ICAM1, intercellular adhesion molecule-1; LEPFC, lipid-encapsulated perfluorocarbon; USPIO, ultra-small superparamagnetic particles of iron oxide.

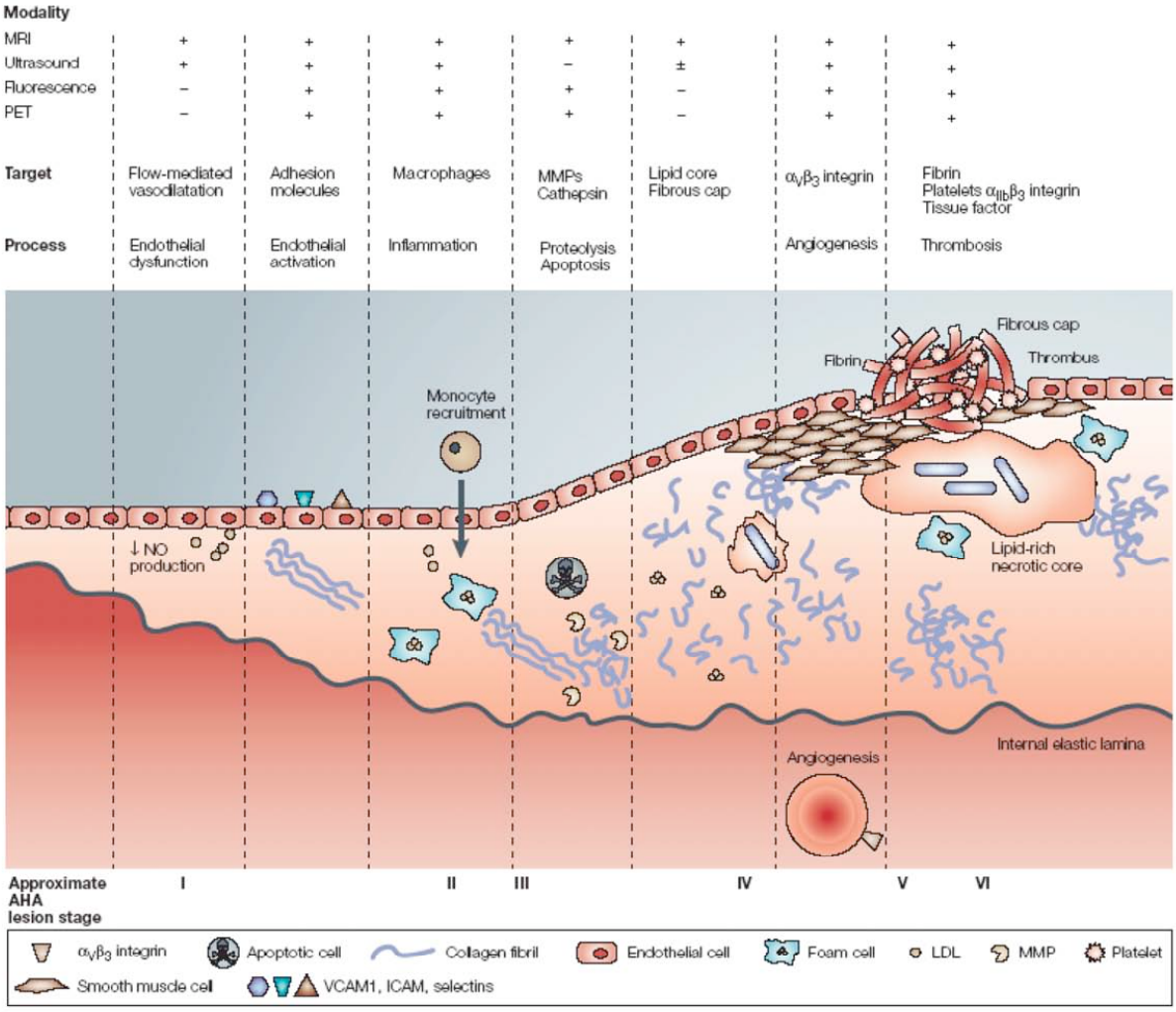

Figure 1: Illustration of processes of atherogenesis ranging from pre-lesional endothelial dysfunction (left) through monocyte recruitment to the development of complicated plaques complicated by thrombosis (right). The mechanisms are grossly simplified but focus on components (e.g. cell adhesion molecules, macrophages, connective tissue elements, lipid core, fibrin) and processes (e.g. apoptosis, proteolysis, angiogenesis, thrombosis) in plaque that have been imaged or that present useful potential imaging targets. Symbols indicate the feasibility (+ or -) of imaging using each of the modalities listed. From Choudhury RP, Fuster V, Fayad ZA. Molecular, cellular and functional imaging of atherothrombosis. Nat Rev Drug Discov. 2004 Nov;3(11):913-25.

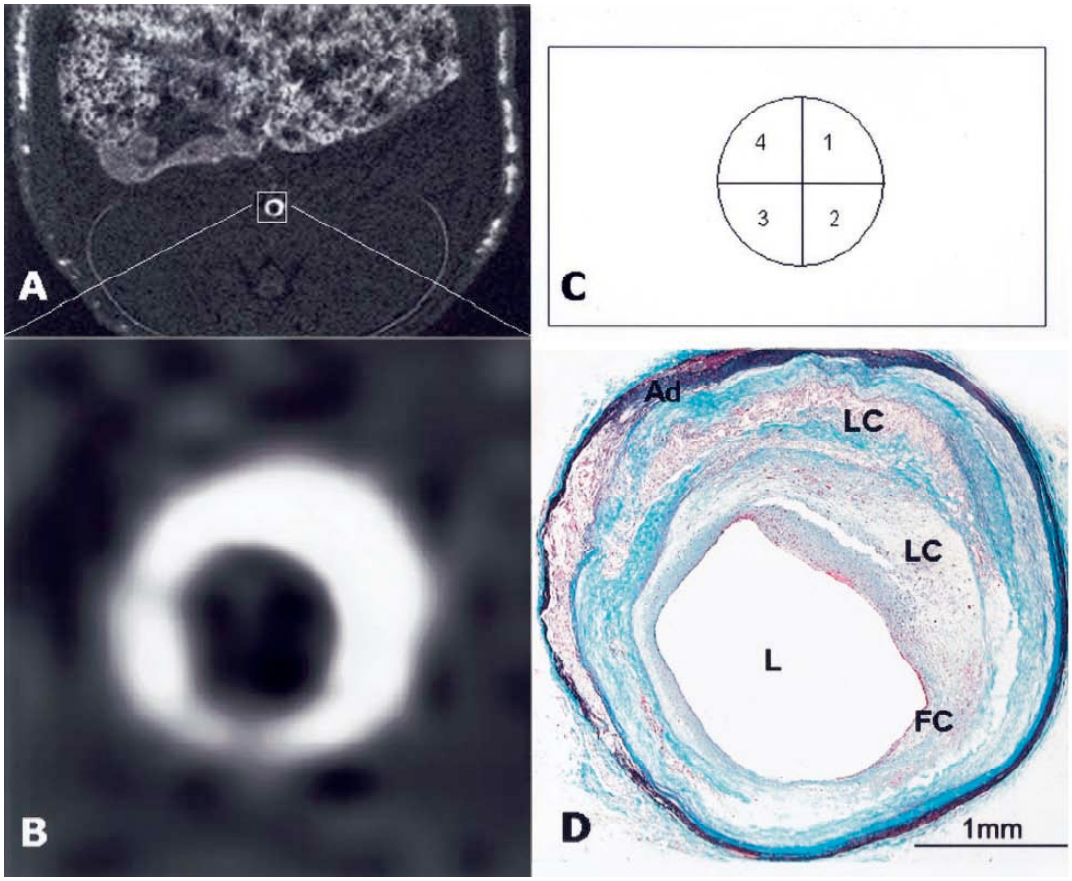

Figure 2: In vivo T1-weighted MR transverse image of atherosclerotic rabbit abdominal aorta 24 hours after gadofluorine injection with the use of IR-DIFF-TFL (A). Magnified panel (B) shows plaque enhancement after injection. Corresponding histopathological section is shown in D. The combined Masson's trichrome elastin staining allows characterization of different plaque components. MR images and histopathological sections were divided into 4 quadrants for further analysis (C). The appearance of MR image correlates closely with the matched histopathological section shown in D. Ad indicates adventitia; FC, fibrous cap; L, lumen; and LC, lipid core.

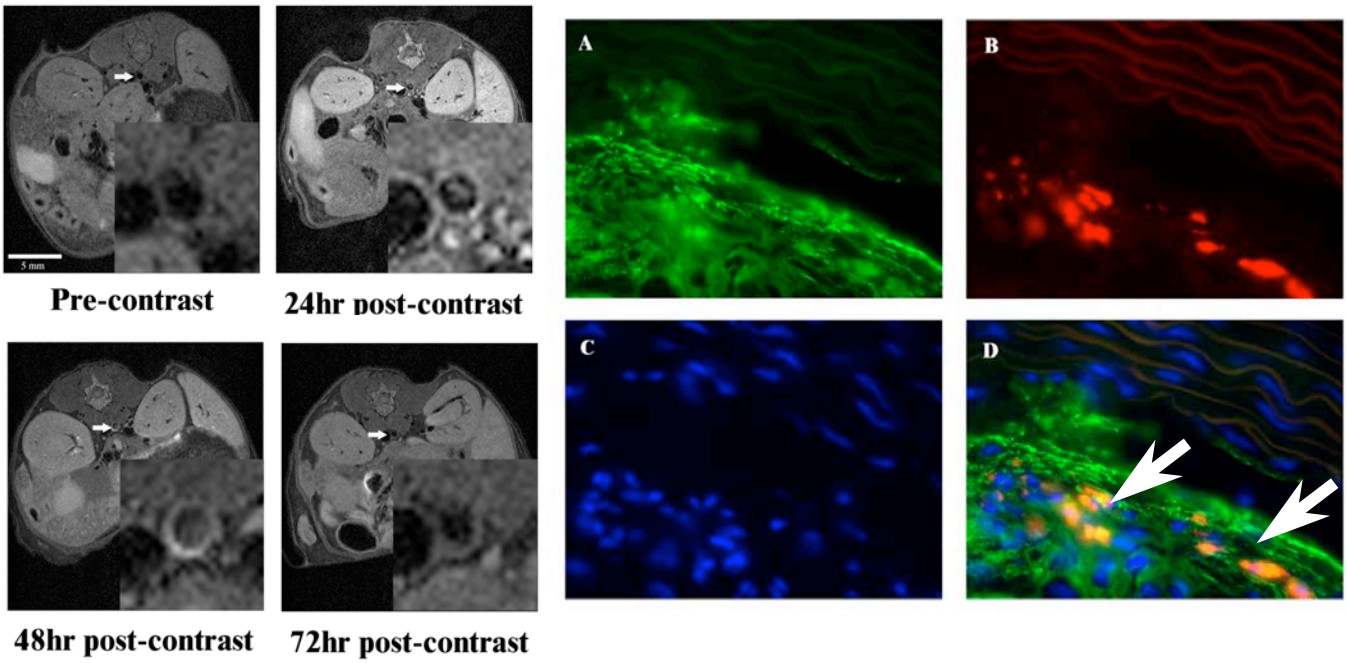

Figure 3: Left. MR pre and post contrast images on the same ApoE knockout mouse at different time-points. Right. Immunofluorescence of ApoE knockout mouse at 24 hours post injection showing co-localization of Gadofluorine M within the extracellular matrix. (A) fluorescently labeled antibody to tenascin for extracellular matrix; (B) carbocyanin labeled Gadofluorine M; (C) DAPI staining for cell nuclei and (D) combined RGB image.

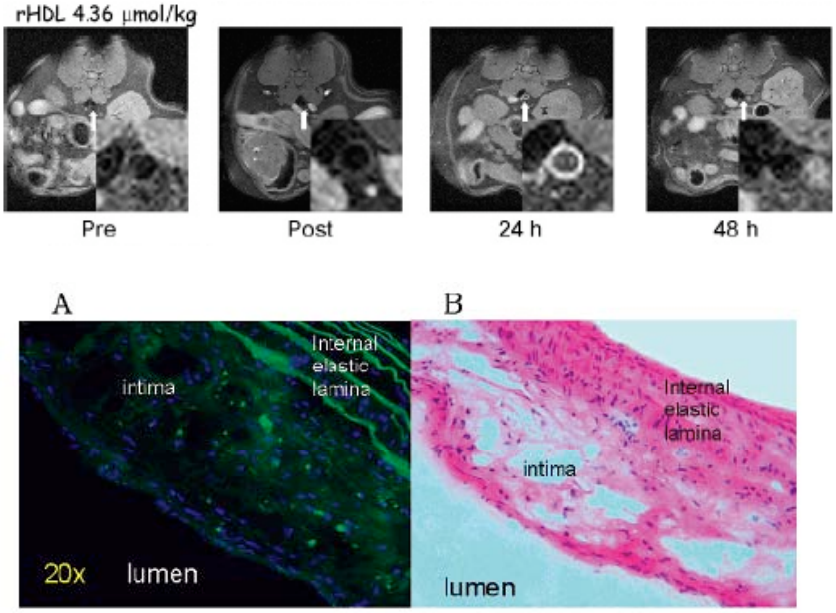

Figure 4: Above. In vivo MRI at different time points (pre- and post injection of contrast agent and at 24, and 48h with the dosage of Gd as determined by ICP-MS measurement. White arrows point to the abdominal aorta; the insets denote a magnified aorta. **Below.** (A) confocal fluorescence microscopy of an atherosclerotic plaque. Blue denotes nuclei (DAPI staining), and green denotes rHDL NBD labeled. (B) Histopathological section stained with hematoxylin and eosin (H&E).

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